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Minnesota Center for Environmental Advocacy

The legal and scientific voice protecting and defending Minnesota's environment

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April 14, 2009

Ms. Angela Preimesberger
Minnesota Pollution Control Agency
520 Lafayette Road North
Saint Paul, MN 55155-4194

Re: Planned Amendments to Rules Governing Water Quality, *Minnesota Rules* Chapters 7050 and 7052

Dear Ms. Preimesberger,

Attached are further comments of the Minnesota Center for Environmental Advocacy (MCEA) in response to the Minnesota Pollution Control Agency's February 19, 2009 Request for Comments. These comments and attached scientific studies are in response to the request for technical information and data related to:

- II.A. Eutrophication standards for river systems, and
- II.C. New or revised contaminant standards for protection of aquatic life and human health from toxic effects—Nitrate

These comments were prepared for MCEA by Dr. Candice Bauer, (Curriculum Vitae also attached).

Thank you for the opportunity to submit these comments.

Sincerely,

Kris Sigford
Water Quality Director

List of Attachments:

- MCEA's Comments by Dr. Candice Bauer
- Curriculum Vitae for Dr. Bauer
- Cited Literature
  - Alonso and Camargo 2008
  - Christensen 2007
  - Dodds and Oakes 2005
  - Shinn and Serrano 2008
  - Stanley and Maxted 2008
  - Tomasso and Grosell 2005

Minnesota Center for Environmental Advocacy Comments and Technical Materials

Eutrophication Standards for River Systems and Nitrate Aquatic Life Toxicity
Submitted to Minnesota Pollution Control Agency Pursuant to
February 19, 2009 Request for Comments

Prepared by Candice R. Bauer, Ph.D.
April 14, 2009

I. Eutrophication Detrimentally Impacts Uses of Minnesota Rivers and Streams.

Excess phosphorus and nitrogen loads to rivers and streams can lead to impairment of beneficial uses including aquatic life, recreation, and drinking water. As such, the Minnesota Pollution Control Agency (MPCA) should evaluate potential criteria values to determine, at a minimum, if they are protective of these designated uses.

Impairment of Aquatic Life Use.
Increased concentrations of nutrients (phosphorus and nitrogen, either alone or in combination) may affect streams and rivers including: (1) increased plant growth termed primary production; (2) increased microbial growth, including bacteria and fungi, termed secondary production; and (3) altered plant types or plant community structure including promotion of cyanobacterial species. Increased phosphorus and nitrogen delivery both work through these same pathways to initiate measurable changes to the biological communities in rivers and streams, as both phosphorus and nitrogen (separately and together) have the potential to increase productivity or alter plant communities in these waters. These changes, including their effect on dissolved oxygen and pH regimes in flowing waters, lead to decline in the integrity or health of macroinvertebrate and fish communities.

Impairment of Recreational and Aesthetic Uses.
Excessive algae as a result of human-caused nutrient enrichment can impair recreational use by decreasing the aesthetic quality of rivers, impairing the enjoyability and feasibility of primary and secondary contact recreation, and making primary contact recreation dangerous due to exposure to cyanobacterial toxins.

Impairment of Drinking Water Use.
Additionally, excessive nutrients can lead to impairment of drinking water use through algal caused decline in drinking water quality through an increased incidence of taste and odor problems and/or disinfection byproducts. Adoption of appropriately protective numeric phosphorus, nitrogen, and algal criteria should minimize the potential for impairment of drinking water uses.

II. Phosphorus Criteria are Needed for Minnesota Rivers to Prevent Impairment of Uses in Minnesota and Downstream Waters.

Excessive Total Phosphorus Concentrations Cause Degradation to Aquatic Life.
River studies conducted by MPCA and peer-reviewed studies from Wisconsin show degradation of aquatic life in rivers as a function of human-caused increases in phosphorus concentrations. MPCA studies, like those conducted in Wisconsin, have demonstrated increased concentrations

of suspended algae as a result of increased phosphorus concentrations and significant correlations between numerous macroinvertebrate and fish metrics and phosphorus concentrations where degraded biological integrity is correlated with increased phosphorus concentrations. More specifically, Robertson et al. 2008 demonstrated that significant thresholds in biological metric scores occur as concentrations increase above reference concentrations of total phosphorus (TP) (around 0.040 mg/L TP) up to 0.150 mg/L TP (as summarized in Table 21), with an average threshold concentration of 0.1 mg/L TP in Wisconsin rivers. Specifically, suspended algal concentrations are significantly greater at TP concentrations greater than 0.064 mg/L and common biological indices (macroinvertebrate HBI and fish IBI) show that significant declines in common measures of biological integrity are common at TP concentrations greater than 0.150 and 0.139 mg/L, respectively. Additionally, the New York State Department of Environmental Conservation (NYDEC) is finalizing a report on its efforts to define the detrimental effects of excessive nutrients on algae and macroinvertebrates in large rivers in Aggregate Ecoregions VII and VIII.[1] As such, MPCA should contact NYDEC staff to obtain the results of the NYDEC river studies. These results should be used as lines of evidence for criteria derivation in Minnesota rivers, particularly in waters draining Aggregate Ecoregions VII and VIII (Minnesota's Northern Lakes and Forests, Northern Minnesota Wetlands, Driftless Area, and North Central Hardwood Forest ecoregions). Further analysis of Minnesota data for determination of similar relationships should be conducted, and weighed in combination with results of the Wisconsin and pending New York studies, to derive Minnesota river TP criteria that are protective of aquatic life uses. (A summary of potential reduction targets for TP is shown in Figure 1.)

TP Eutrophication Criteria Should Meet Downstream Use Goals.
In accordance with 40 CFR § 131.10 (b), Minnesota "shall take into consideration the water quality standards of downstream waters and shall ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." In Minnesota, downstream waters include, but are not limited to, Lake Pepin, Lake Winnipeg, Lake Superior, and the Gulf of Mexico. As such, MPCA shall ensure that the water quality standards (including narrative and numeric criteria and designated uses) of downstream waters are protected when river nutrient criteria for Minnesota are adopted.

Lake Pepin: Lake Pepin, a natural reservoir on the Mississippi River, has been detrimentally impacted by excessive nutrients and sedimentation for which MPCA has included Lake Pepin on its 303(d) list of impaired waters due to impairment of its designated uses. Of particular concern are high levels of algae, resulting from excessive nutrient concentrations. MPCA recently adopted lake nutrient eutrophication standards, including TP criteria. Application of the lake eutrophication standards to Lake Pepin (it is a deep lake, with a maximum depth greater than 15 feet, and is located in the Western Cornbelt Plains ecoregion) would set a TP criterion of 65 ug/L and a chlorophyll *a* criterion of 22 ug/L. Since Lake Pepin has sizeable drainage from the North Central Hardwood Forests ecoregion, one could also consider the eutrophication standards for that ecoregion, 40 ug/L TP and 14 ug/L chlorophyll *a* concentrations, as potential criteria necessary for protection of Lake Pepin's designated uses. Although the lake eutrophication standards allow the adoption of site-specific criteria for reservoirs, they do not preclude use of the lake standards for reservoirs like Lake Pepin.

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[1] A.J. Smith, New York State Department of Environmental Conservation, Stream Biomonitoring Unit, Troy, NY, personal communication, Feb. 23, 2009. Use of a weight of evidence approach to define nutrient criteria protective of aquatic life (in preparation).

Site-specific studies to determine acceptable concentrations of nutrients and algae have been conducted. A 1995 published study of Lake Pepin by MPCA staff estimated that in-lake concentrations of 70 ug/L were necessary to restore the uses of Lake Pepin (Heiskary and Walker 1995). In contrast, current efforts being undertaken by MPCA recommend that in-lake TP concentrations be restored to 100 ug/L (Heiskary 2008), although this management objective has not been formally incorporated into an U.S. Environmental Protection Agency (USEPA)-approved TMDL or water quality standard. Using the range of potential in-lake TP concentrations outlined (40 to 100 ug/L TP), TP concentrations in major tributaries to Lake Pepin (like the Mississippi, Minnesota, St. Croix, and Cannon Rivers) must be reduced to levels that will protect the downstream uses in Lake Pepin. Since inflow TP concentrations strongly influence in-lake TP concentrations (Heiskary 2008), TP criteria for rivers flowing into Lake Pepin must not exceed Lake Pepin's approved in-lake TP criterion. Alternatively, MPCA must provide a determination that tributary river TP criteria, as supported by scientifically-defensible modeling, ensure protection of the in-lake criterion.

Lake Winnipeg: Lake Winnipeg is suffering from excessive algal blooms including massive blue-green algal proliferations and related detrimental impacts to aquatic life associated with excessive nitrogen and phosphorus loadings from the watershed. The Lake Winnipeg Action Plan calls for nutrient inflows to the lake to be restored to pre-1970 levels, with inflow concentrations of TP from the southern basin returning to concentrations between 50 and 100 ug/L.[2] Reducing phosphorus inputs from the Red River, which delivers approximately 60% of the total phosphorus load of Lake Winnipeg, will be especially important to restoring water quality and protecting Lake Winnipeg as a downstream water of the Red River. As such, water quality must be restored in Minnesota waters that drain to the Red River (primarily Level 3 ecoregion 48, identified by MPCA as the Red River Valley ecoregion) and subsequently to Lake Winnipeg. U.S. Geological Service (USGS) reported that the median concentration of TP in the Red River near the Canadian border is approximately 200 ug/L (Christensen 2007). Therefore, the concentration of TP in the U.S. portion of the Red River must be reduced between 50 to 75% in order to meet the Lake Winnipeg restoration goal of Red River instream TP concentrations between 50 and 100 ug/L. In general, tributaries to the Red River would, in the least, have to restore water quality to a level that approximates the 25th percentile of concentrations reported for all waters in Ecoregion 48 in order to prevent the impairment of Lake Winnipeg uses. Nutrient criteria for the Red River should be set at 100 ug/L (1970's estimate of water quality in the Red River), or a level shown by water quality modeling to allow for TP concentrations in Lake Winnipeg to meet its uses.

Gulf of Mexico: USEPA's science advisory board calls for a 45% reduction in total phosphorus delivery to Gulf waters in order to promote restoration of the Gulf and protect Gulf uses (USEPA Science Advisory Board 2007). Consideration of how Minnesota will meet this goal must be included in the criteria derivation process. Total phosphorus criteria to control eutrophication of Minnesota, or other downstream waters, may be more stringent than controls necessary to meet the 45% Gulf of Mexico reduction goal. However, at a minimum, criteria should ensure that Minnesota, when its waters attain phosphorus criteria, will have done its part toward meeting the goals of the downstream uses in the Gulf of Mexico in accordance with USEPA regulations at 40 CFR § 131.10 (b). As demonstrated in Figure 1, TP may need to be reduced to mean

[2] Materials on the Lake Winnipeg Action Plan available online at:
http://www.gov.mb.ca/waterstewardship/water_quality/lake_winnipeg/action_plan.html

concentrations near the 25th percentile of TP for all waters in each ecoregion to meet an overall reduction goal of 45% in Minnesota waters (as calculated from median concentrations reported for Minnesota ecoregions in USEPA 2001). This suggests that a 45% reduction in TP is attainable in Minnesota, but since some northern ecoregions have TP concentrations that are currently near the estimated reference or pre-European concentrations, attaining this goal would require a greater than 45% reduction target where existing TP concentrations are much greater than the estimated reference concentrations, including the Western Cornbelt Plains, Northern Glaciated Plains, and the Driftless Area.

Lake Superior: Minnesota rivers that discharge to Lake Superior should be evaluated for their contribution to nearshore and open lake nutrient and algae concentrations. In no way should these rivers cause or contribute to an exceedence of the International Joint Commission target for Lake Superior (5 ug/L),[3] or cause or contribute to impairment of designated uses for nearshore areas, bays, or estuaries.

III. Nitrogen Criteria are Needed for Minnesota Rivers to Prevent Impairment of Uses in Minnesota and Downstream Waters.

Increased concentrations of nitrogen in aquatic ecosystems have resulted from human activities in Minnesota watersheds. These excessive nitrogen concentrations are of concern because of (1) the stimulation of production in Minnesota's riverine algal and plant communities and changes to plant community composition; (2) the cascading effects of nutrients to macroinvertebrates and fish as a result of changes to plants and algae; (3) potential lethality of environmentally relevant inorganic nitrogen to resident macroinvertebrate and amphibian species; and (4) its transport to downstream waters contributes significantly to the presence of hypoxia in the Gulf of Mexico. These effects should be addressed through the proposal of total nitrogen (TN) eutrophication criteria for rivers that prevent excessive algal proliferations and detrimental impacts to resident biota, as well as preventing impairment of downstream uses as required by USEPA regulations at 40 CFR § 131.10 (b), and the adoption of nitrate and/or nitrite criteria to prevent toxic impacts of these components of nitrogen. (Figure 2 shows potential reduction target for total nitrogen.)

Excessive nitrogen leads to eutrophication of Minnesota rivers.
MPCA should carefully analyze available data on the effects of excessive nitrogen on the resident aquatic life in Minnesota rivers, as well as relevant data collected in nearby waters such as the Wisconsin nonwadeable river report (Robertson et al. 2008). Information from Wisconsin suggests that, on average, TN greater than 1 mg/L (ranging from about 0.6 mg/L to 2 mg/L) has significant detrimental impacts on aquatic life and details how nitrogen concentrations exceeding reference concentrations lead to increased algae and decreased biological integrity of macroinvertebrate and fish communities living in the affected waters. Furthermore, Montana studies in the Northern Glaciated Plains ecoregion found that TN greater than 1.12 mg/L led to decreased quality of algae and indicated decreased minimum dissolved oxygen concentrations were present in enriched waters.[4] This information, in conjunction with data from Minnesota further supports the need to adopt TN criteria to prevent impairment of the aquatic life use of

[3]Great Lakes targets are cited in the (draft) State of the Great Lakes Report 2009 available at: http://www.solecregistration.ca/documents/0111%20Phosphorus%20Concentrations%20and%20Loadings%20(SOLEC%202008).pdf
[4]Available at: http://www.deq.state.mt.us/wqinfo/Standards/WhitePaper_FNL3_Nov12-08.pdf

Minnesota rivers. Specifically, MPCA has found that increased TN concentrations are correlated with increased algae and biological oxygen demand, as well as declining fish and macroinvertebrate biological metric scores.

TN Eutrophication Criteria Should Meet Gulf of Mexico TN Reduction Goals. USEPA's science advisory board calls for a 45% reduction in total nitrogen delivery to Gulf waters in order to promote restoration of the Gulf and protect Gulf uses (USEPA Science Advisory Board 2007). Consideration of how Minnesota will meet this goal must be included in the criteria derivation process. While total nitrogen criteria to control eutrophication of Minnesota waters may be more stringent than controls necessary to meet the 45% reduction goal, at a minimum, criteria should ensure that Minnesota, when its waters attain nitrogen criteria, will have done its part toward meeting the goals of the downstream uses in the Gulf of Mexico in accordance with USEPA regulations. As demonstrated in Figure 2, TN may need to be reduced to mean concentrations near the 25th percentile of TN for all waters in each ecoregion to meet an overall reduction goal of 45% in Minnesota waters (as calculated from median concentrations in Minnesota waters included in the national nutrient database). This suggests that a 45% reduction in TN is attainable in Minnesota, but again, since some northern ecoregions currently have TN concentrations near the estimated reference or pre-European concentrations, a greater than 45% reduction target may be needed in the Western Cornbelt Plains, Northern Glaciated Plains, and Driftless Area.

Additionally, MPCA should evaluate whether TN criteria are necessary to provide for the attainment and maintenance of the water quality standards of other downstream waters, including (but not limited to) Lake Pepin, Lake Winnipeg, and Lake Superior. In particular, MPCA should evaluate the role of TN in promotion of cyanobacteria and cyanobacterial-caused impairment of drinking water and recreational designated uses.

Nitrate Criteria are Necessary to Prevent Toxicity to Aquatic Life. Emerging information suggests that high levels of nitrate and nitrite in similar waters may also be a toxicological concern for resident aquatic life (Stanley and Maxted 2008). As such, MPCA's proposal to adopt nitrate criteria is applauded. According to information provided by MPCA staff,[5] MPCA has compiled and reviewed studies related to the toxicological effect of nitrate on aquatic life. From this review, MPCA has found that many of these studies provide information suitable for use in deriving acute and chronic aquatic life water quality criteria for nitrate.

While data obtained by MPCA to date do not meet the specifications for **acute** criteria derivation in accordance with the 1985 aquatic life criteria derivation procedures (data is available for only 6 of 8 required taxa groups), procedures adopted into Minnesota water quality standards as a part of the Great Lakes Initiative (Tier 2 criteria) should be used to calculate water quality criteria based upon the available data. Alternatively, additional acute toxicity data, if available, could be added to the existing database to allow for the calculation of a nitrate criterion in accordance with the 1985 guidelines.

Regarding data available to calculate a **chronic** nitrate criterion, MPCA has found that data is available to calculate an acute to chronic ratio (ACR). The current data suggests that the ACR

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[5] Steve Heiskary, personal communication, January 12, 2009, and Phil Monson, personal communication, February 9, 2009.

for nitrate is high relative to ACRs for other toxicants. Because of the importance of the ACR to the calculation of the chronic criterion value and the chronic criterion's importance to the water quality management program (identification of impaired waters, subsequent TMDLs, and NPDES permit decisions), MPCA should carefully scrutinize the available data for determination of an appropriate ACR and provide sufficient justification to support the ACR calculation. New data on the chronic effects of nitrate on aquatic life would be helpful, although not necessary, in providing justification of any proposed chronic nitrate criteria. MPCA should coordinate with USEPA Region 5 Water quality standards staff to ensure that all available and relevant data, including any new data that may be collected prior to publication of proposed nitrate criteria, are used for criteria derivation purposes.

Information on the Toxicity of Nitrite to Aquatic Life Should be Analyzed.
Nitrite is also toxic to aquatic organisms. While neither USEPA nor MPCA has, to our knowledge, compiled and analyzed the nitrite aquatic life toxicity database, further efforts should be made to do so. Information from Wisconsin streams and rivers have shown that instream concentrations of nitrite are greater than previously thought and could reach levels that are toxic to some forms of aquatic life (Stanley and Maxted 2008). MPCA should further investigate the presence and levels of nitrite in Minnesota waters, as well as currently available (*see* Tomosso and Grossel 2005, Alonso and Camargo 2008, Shinn et al. 2008, and references within) and/or new data on nitrite toxicity. This investigation should then conclude whether or not sufficient data is available for criteria derivation at this time, and if not, specify additional data needs.

From a water quality management perspective, total nitrogen, nitrate, and nitrite concentrations in rivers and streams are all inter-related. Therefore, water quality management decision-making should consider the local and downstream impacts of excessive nitrogen in Minnesota waters to eutrophication and toxic impacts to aquatic life in making waterbody assessments and NPDES permit decisions.

IV. Numeric Algal Abundance (Measured as Chlorophyll *a*) Criteria are Necessary to Protect Recreational and Aesthetic Uses and Implement the Narrative Prohibition of Nuisance Conditions.

Numeric nuisance algal criteria (expressed as the amount of algae present on stream or river bottoms) are under development for protection of recreational use (as a link to aesthetic quality) for rivers in Montana.[6] Development of a similar nuisance algal water quality criterion to prevent excessive amounts of benthic algae in Minnesota, in addition to eutrophication criteria for phosphorus and nitrogen, would provide a comprehensive strategy to protect Minnesota waters from human-caused eutrophication. Because nutrients can cause numerous effects in flowing waters, impairment of designated uses can occur even when nuisance algal growths are not present or are present infrequently. As such, numeric criteria for TN and TP should apply independent of numeric algal criteria. Adoption of an independent numeric nuisance algal criterion would allow assessment of nutrient impairment in any streams that are not covered by numeric nutrient criteria adopted through this rulemaking, and may help in the derivation of nutrient criteria for these streams in the future.

[6] A description of this study is available at http://www.deq.state.mt.us/wqinfo/Standards/WhitePaper_FNL3_Nov12-08.pdf.

V. Characteristics of Protective Eutrophication Water Quality Standards.

Eutrophication standards should protect sensitive, existing, designated, and downstream uses. A viable procedure to determine the appropriate level of protection for each water should weigh information on the current nutrient concentrations, designated use of the water, and information on criteria concentrations necessary to protect downstream uses, aquatic life endpoints, and potentially sensitive recreational, drinking water, or high quality water uses. To address these issues, criteria should be modeled upon the following principles.

Protection of Existing Uses.
In aquatic ecosystems, including rivers, waters with low nutrient concentrations are recognizably different from eutrophic, or nutrient-rich, waters. The community of aquatic organisms present in waters that have not suffered from cultural eutrophication cannot, and do not, exist in waters that have undergone sustained and significant increased nutrient loadings due to increased human activities on the landscape. These changes can be profound and biologically meaningful, such that the end result is a degraded or impaired aquatic life use for the water. Generally, it has been found that these changes in aquatic communities begin to occur in response to relatively small increases of nutrients in a water. This was found in Montana and New York analyses looking at a comparison of stressor-response relationships and reference conditions.[7] For example, the quantity of algae can increase, the types of algae change, and sensitive macroinvertebrate and fish species begin to decline. As such, it will be imperative that any proposed eutrophication criteria provide a level of protection that will ensure the protection of existing uses (a low-nutrient, high-quality aquatic community). This could be accomplished by setting a stringent criterion to protect the quality of these waters (equivalent to concentrations less than or equal to the lowest significant threshold(s) of biological change found for Minnesota or nearby waters or equal to or less than reference concentrations approximating a pre-European condition). Alternatively, MPCA could adopt antidegradation policies that prevent additional loading of nutrients to such waters in order to ensure that existing uses are adequately protected. For example, any water with nutrient concentrations at or below designated "reference" concentrations would have a criterion equal to the reference concentration (sensu Sorrano et al. 2007 approach for protection of reference-quality lakes in Michigan).

Protection of Designated Uses.
For other "general" aquatic life use waters, criteria should be derived to prevent changes to the aquatic life at a level of protection tailored to the attainment of designated uses (i.e., coldwater or warmwater fishery). Close coordination with MPCA staff working on biological criteria development and close scrutiny of all available information from Minnesota and nearby waters (specifically, Wisconsin) on the relationship between increased nutrient concentrations and degradation of biological condition, as well as all available information on the level of protection necessary for downstream uses, is necessary to adequately evaluate potential nutrient endpoints for criteria derivation purposes.

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[7] http://www.deq.state.mt.us/wqinfo/Standards/WhitePaper_FNL3_Nov12-08.pdf and A.J. Smith (NYDEC), personal communication, respectively.

Suggested River Eutrophication Standards Rule Language.

A. For high quality rivers and streams, whose phosphorus concentrations are less than or equal to estimated reference total phosphorus (0.04 mg/L) and total nitrogen (0.6 mg/L) concentrations, the criteria for those waterbodies will be set at the applicable estimated reference phosphorus concentration or at current nutrient concentrations. Please note that reference values included here are derived based upon the highest average estimated reference (pre-European) condition for Minnesota level III ecoregions from Robertson et al. 2008, Dodds and Oakes 2004, and Smith et al. 2003 (as cited by Dodds and Oakes 2004). However, reference concentrations of nutrients could be calculated for each ecoregion independently or via alternative methods. If reference nutrient concentrations are different from the levels stated in Parts A, B, and C, MPCA should alter the suggested criteria values in these parts accordingly and provide sufficient justification to support the change.

B. For drinking waters and trout waters, total phosphorus concentrations shall be less than or equal to 0.04 mg/L and total nitrogen shall be less than 0.6 mg/L in order to protect and restore water quality in these high quality waters, regardless of the current nutrient concentrations, unless nutrient-response relationships are found to support the adoption of alternative nutrient criteria.

C. For waters with current nutrient concentrations greater than reference concentrations (0.04 mg/L TP and 0.6 mg/L TN), criteria will be derived by evaluating current nutrient concentrations and identified significant biological thresholds. Specifically, if current nutrient concentrations are greater than reference concentrations and there are two or more biological thresholds proposed for criteria derivation purposes, the site's nutrient criteria is set at next higher biological threshold value (for protection of existing uses and antidegradation policies). If current condition is greater than the highest biological threshold, the criterion is set equal to the highest biological threshold value and the water is listed for impairment of designated uses. (Note: One could adapt this approach to also consider ecoregion in making criteria determinations such that reference and/or biological threshold values are unique to each ecoregion.)

For example, Wisconsin found that significantly increased concentrations of suspended algae were present when TP concentrations exceeded 0.064 mg/L TP and TN concentrations exceeded 0.9 mg/L TN, while macroinvertebrate and fish metric scores showed significant decline in biological health at concentrations, on average, greater than 100 ug/L TP and 1 mg/L TN. Therefore, these biological thresholds would be used for criteria derivation for individual Minnesota rivers and criteria would be assigned as follows:

Current Condition (cc)	Proposed Criterion
Less than or equal to 0.04 mg/L TP Less than or equal to 0.6 mg/L TN	0.04 mg/L TP 0.6 mg/L TN
0.04 mg/L TP $<$ cc $\leq$ 0.065 mg/L TP 0.6 mg/L TN $<$ cc $\leq$ 0.9 mg/L TN	0.065 mg/L TP 0.9 mg/L TN
0.065 mg/L TP $<$ cc $<$ 0.1 mg/L TP 0.9 mg/L TN $<$ cc $<$ 1.0 mg/L TN	0.1 mg/L TP 1 mg/L TN
cc $>$ 0.1 mg/L TP cc $>$ 1 mg/L TN	0.1 mg/L TP (listed as impaired water) 1 mg/L TN (listed as impaired water)

Using this approach would set stringent, reference-based criteria for numerous waters in the Northern Lakes and Forests, Northern Minnesota Wetlands, and North Central Hardwood Forests ecoregions where reference-based criteria suggested here are near the 25th percentile values for all waters (i.e., 25% of waters would be protected by these more-stringent criteria), while other waters would receive criteria based upon identification of nutrient concentrations that lead to significant biological change (i.e., significant increase in algae, decline in sensitive fish species or macroinvertebrate richness). In addition, these criteria could be applied to waters of special ecological significance (remaining low-nutrient waters) in the other ecoregions although few waters would be expected to meet reference criteria due to substantial human impacts to the landscape.

Alternative Approach to Criteria Derivation.
We encourage MPCA to continue analysis of its data and other relevant studies to determine the phosphorus and nitrogen concentrations that lead to decline in biological integrity for use in deriving criteria that are protective of aquatic life uses. In the event that statistically and biological significant relationships between biological measures and nutrient concentrations are not clearly identified through this analysis, criteria for all waters could be set at a level equivalent to minimally-impacted conditions, although defining this for Minnesota rivers may require additional data collection or modeling to accurately define nutrient criteria using this methodology. Setting the criteria equal to minimally-impacted conditions assumes that the existing and designated uses are protected for all waters since they are in a "pristine" or natural state and detrimental effects are only cause for impairment of uses when concentrations are increased over this level (sensu Montana's approach). Or, criteria could be set based upon studies conducted in waters of Aggregate Ecoregions VI, VII, and VIII, particularly those conducted in Michigan, Wisconsin, Montana, and New York.

Literature Cited.

Alonso, A. and J.A. Camargo. 2008. Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: a comparative study of amphipods and planarians. Archives of Environmental Contamination and Toxicology 54:259-265. (Attached)

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U.S. Environmental Protection Agency Science Advisory Board. 2007. Hypoxia in the Northern Gulf of Mexico. Available at: http://epa.gov/msbasin/pdf/sab_report_2007.pdf.

Figure 1.
Current median (yellow bars) and 25th percentile (blue diamonds) total phosphorus concentrations in Minnesota's flowing waters as calculated from data contained in USEPA's nutrient criteria database as compared to reference concentrations (blue triangles; average of values reported by Dodds and Oakes (2004) and Robertson et al. (2008)). Also, potential target criteria values for protection of downstream uses are represented by pink squares (45% reduction in median concentration in ecoregions draining to the Mississippi River), red circles (average concentration reported as necessary for protection of Lake Pepin uses in Heiskary and Walker 1995 and Heiskary 2008), and purple X (average concentration reported as necessary for Red River TP concentrations to restore Lake Winnipeg as suggested by Action Plan). Potential target criteria values for protection of instream uses are represented by blue diamonds (reference concentrations) and red crosses (average instream TP concentration determined by the Wisconsin Department of Natural Resources to be protective of aquatic life in Wisconsin rivers).

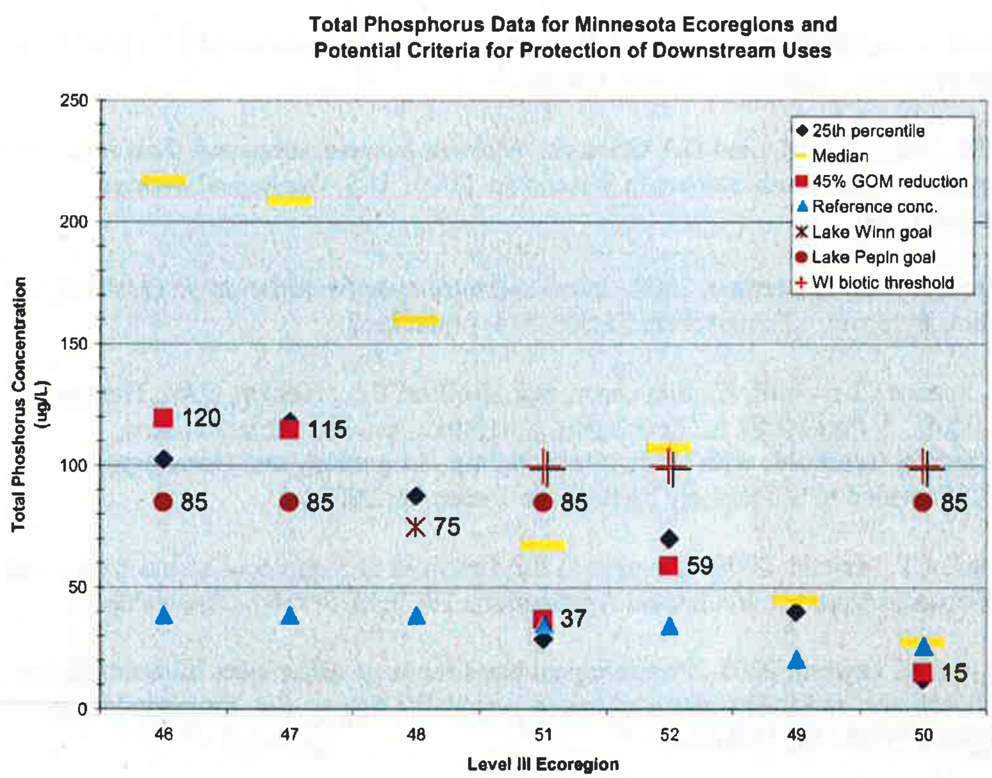
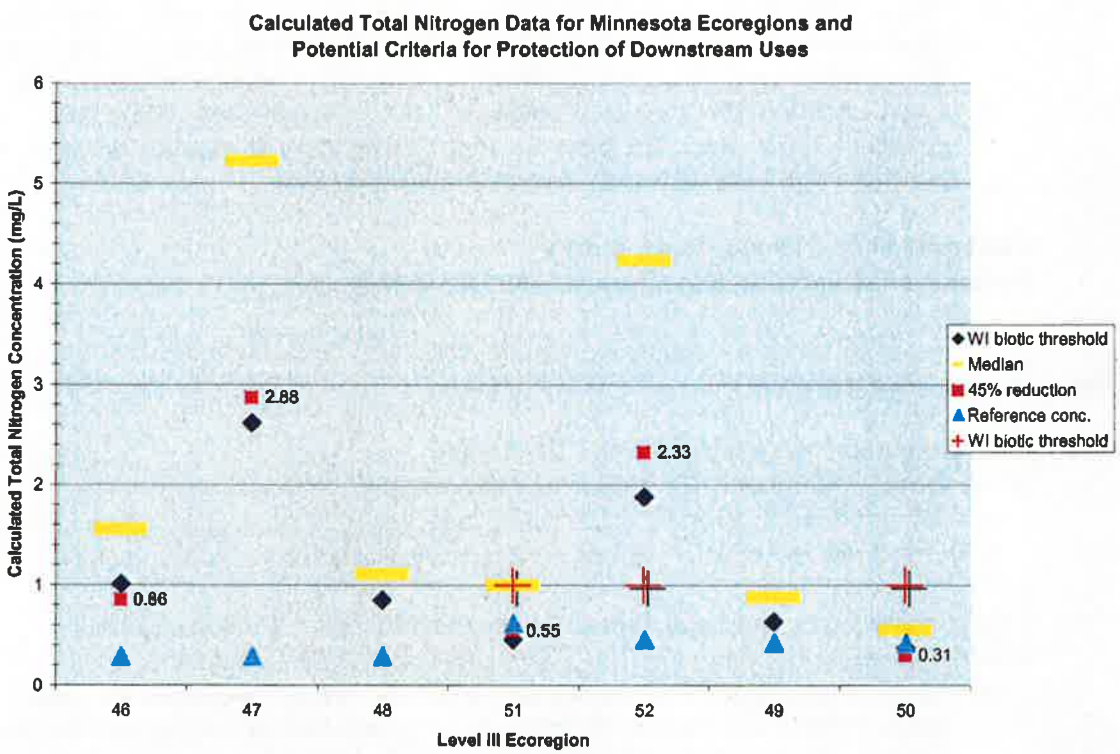


Figure 2.
Current median (yellow bars) and 25th percentile (blue diamonds) of calculated total nitrogen concentrations in Minnesota's flowing waters as calculated from data contained in USEPA's nutrient criteria database as compared to reference concentrations (blue triangles; average of values reported by Dodds and Oakes (2004) and Robertson et al. (2008)). Also, potential target criteria values for protection of downstream uses are represented by pink squares (45% reduction in median concentration in ecoregions draining to the Mississippi River). Potential target criteria values for protection of instream uses are represented by blue diamonds (reference concentrations) and red crosses (average instream calculated TN concentration determined by the Wisconsin Department of Natural Resources to be protective of aquatic life in Wisconsin rivers).



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- Claire Luce Booth Fellowship for Women in Science, August 1998 – May 2003
- Academic Press Award Recipient for Best Student Paper in Applied Sciences, North American Benthological Society Annual Meeting, May 2001

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Bachelor of Science, Biology, Summa Cum Laude, May 1998

PROFESSIONAL EXPERIENCE

Environmental Consultant, Libertyville, Illinois
Water Quality Standards Development, October 2006 – Present

- Advise clients on scientific and technical issues related to development of nutrient criteria
- Provide recommendations related to water quality standards and implementation

United States Environmental Protection Agency, Region 5, Chicago, Illinois
Nutrient Criteria Coordinator (Life Scientist), March 2001 – September 2006

- Managed regional nutrient criteria program including: recipient selection and grant management of EPA funding, participation in and leadership among National Nutrient Criteria Team personnel, leadership in strategic planning for National Nutrient Criteria Team and National Nutrient Criteria Implementation Workgroup, review of nutrient criteria rulemaking information and technical reports
- Planned and coordinated meetings among EPA, state and academic community partners, and stakeholders to facilitate information sharing, as well as moderation and facilitation of such meetings
- Reviewed, and recommended for approval, water quality rule submissions by state and tribal water quality agencies in accordance with the Clean Water Act and Endangered Species Act
- National Award Nominee (Regional Administrator's Award for Excellence Recipient) for having an exceptional impact on national water quality standards policies in the areas of the Endangered Species Act and the development of nutrient criteria, March 2005
- Certificates of Completion: Basic Water Quality Standards Academy (March 2002), NPDES Permit Writer's Training Course (August 2005)

University of Notre Dame, South Bend, Indiana

Teaching Assistant

- General Ecology (August 1998 – December 1998): Co-taught laboratory sessions including: set-up of laboratory experiments, introductory lectures for laboratory sessions, and grading of laboratory reports
- Introduction to Biostatistical Analysis (January 2000 – May 2000): Co-taught laboratory sessions including: introductory lectures for laboratory (computer) sessions, step-by-step instruction of laboratory exercises using SYSTAT statistical software, participation in pre-exam tutorial sessions, and grading of laboratory reports and lecture exams

Supervisor of Research Assistants

- Instructed and supervised numerous summer and school-year undergraduate research assistants on fish care, experimental set-up and maintenance, data collection and entry, and literature review
- Worked with high school student to co-develop and execute project including: experimental design, data collection and analysis, synthesis of information, and report

PUBLICATIONS

Bauer, C.R., and G.A. Lamberti (*In preparation*). Potential effects of zebra mussels and invasive fish (Eurasian ruffe and round gobies) on yellow perch: Predictions from competition and predation experiments. *Journal of Great Lakes Research.*

Bauer, C.R., A.M. Bobeldyk and G.A. Lamberti (*2006*). Predicting habitat use and trophic interactions of the 'exotic triad', Eurasian ruffe, round gobies, and zebra mussels, in nearshore areas of the Great Lakes. *Biological Invasions (Online First)* 10.1007/s10530-006-9067-8.

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PRESENTATIONS

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Bauer, C.R., and G.A. Lamberti. The response of Great Lakes yellow perch to predation and competition with increasing range and population sizes of round gobies and Eurasian ruffe. North American Benthological Society, Athens, GA, May 2003.

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Bauer, C.R., G.A. Lamberti, and M.B. Berg. Zebra mussels, round gobies, and Eurasian ruffe: Predicting ecological impacts of the 'exotic triad' to improve control. State of Lake Michigan Research Symposium, Muskegon, MI, November 2001.

Bauer, C.R., G.A. Lamberti, and M.B. Berg. Potential interactions between Eurasian ruffe and round gobies in the Great Lakes: Prey and habitat preferences. Annual Meeting of the North American Benthological Society, LaCrosse, WI, June 2001.

Bauer, C.R., G.A. Lamberti, and M.B. Berg. Zebra mussels, round gobies, and Eurasian ruffe: Predicting ecological impacts of the 'exotic triad' to improve control. Illinois-Indiana Sea Grant College Program Research Symposium, Chicago, IL, April 2001.

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Ameliorating Effect of Chloride on Nitrite Toxicity to Freshwater Invertebrates with Different Physiology: a Comparative Study Between Amphipods and Planarians

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Abstract High nitrite concentrations in freshwater ecosystems may cause toxicity to aquatic animals. These living organisms can take nitrite up from water through their chloride cells, subsequently suffering oxidation of their respiratory pigments (hemoglobin, hemocyanin). Because NO_2^- and Cl^- ions compete for the same active transport site, elevated chloride concentrations in the aquatic environment have the potential of reducing nitrite toxicity. Although this ameliorating effect is well documented in fish, it has been largely ignored in wild freshwater invertebrates. The aim of this study was to compare the ameliorating effect of chloride on nitrite toxicity to two species of freshwater invertebrates differing in physiology: *Eulimnogammarus toletanus* (amphipods) and *Polycelis felina* (planarians). The former species presents gills (with chloride cells) and respiratory pigments, whereas in the latter species these are absent. Test animals were exposed in triplicate for 168 h to a single nitrite concentration (5 ppm NO_2-N for *E. toletanus* and 100 ppm NO_2-N for *P. felina*) at four different environmental chloride concentrations (27.8, 58.3, 85.3, and 108.0 ppm Cl^-). The number of dead animals and the number of affected individuals (i.e., number of dead plus inactive invertebrates) were monitored every day. LT_{50} (lethal time) and ET_{50} (effective time) were estimated for each species and each chloride concentration. LT_{50} and ET_{50} values increased with increases in the environmental chloride concentration, mainly in amphipods. Results clearly show that the ameliorating effect of chloride on nitrite toxicity was more significant in amphipods than in planarians, likely because of the absence of gills (with chloride cells) and respiratory pigments in *P. felina*. Additionally, this comparative study indicates that the ecological risk assessment of nitrite in freshwater ecosystems should take into account not only the most sensitive and key species in the communities, but also chloride levels in the aquatic environment.

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Introduction

Nitrite (NO_2^-) is a natural component of the nitrogen cycle in aquatic systems (Wetzel 2001; Philips *et al.* 2002). This ion is an intermediate oxidation form between ammonia (NH_3+NH_4^+) and nitrate (NO_3^-), being able to be found in unpolluted freshwater ecosystems at concentrations between 0.001 and 0.005 ppm NO_2-N (Kelso *et al.* 1997; Wiesche and Wetzel 1998; Wetzel 2001). Nevertheless, point and nonpoint pollution sources (*e.g.*, industrial wastewater effluents, municipal sewage effluents, runoff from agriculture, emissions to the atmosphere and the subsequent atmospheric depositions) have significantly increased nitrite concentrations (as well as the concentrations of ammonia and nitrate) in freshwater ecosystems (Lewis and Morris 1986; Meybeck *et al.* 1989; Gleick 1993; Smil 2001; Philips *et al.* 2002; Camargo and Alonso 2006).

Elevated concentrations of inorganic nitrogen compounds (*i.e.*, ammonia, nitrite, and nitrate) in freshwater ecosystems can induce ecotoxicological processes because they are toxic to aquatic animals (Lewis and Morris 1986; Alcaraz and Espina 1994; Jensen 1995; Philips *et al.* 2002; Beketov 2004; Camargo *et al.* 2005; Camargo and Alonso 2006). In the case of nitrite, its toxicity is better documented to fish than to other aquatic animals (Camargo and Alonso 2006), although freshwater invertebrates can have higher toxicological relevance because they usually show a much wider tolerance range to pollutants (Buikema and Voshell 1993; Camargo and Alonso 2006). Among freshwater invertebrates, planarians and amphipods exhibit high sensitivity to various toxicants (Best *et al.* 1981; Pantani *et al.* 1997; Alonso and Camargo 2004, 2006b). In addition, these two animal groups are important components of the benthic macroinvertebrate communities because they play key trophic roles as predators (planarians) and shredders (amphipods) (Lock and Reynoldson 1976; Cummins and Klug 1979; Thorp and Covich 2001; Alonso and Camargo 2004, 2006a).

Aquatic animals can take nitrite up from water through their chloride cells, located in the gills (fish and crustaceans) and in the anal papillae (*Chironomus* larvae, Diptera) (Lewis and Morris 1986; Alcaraz and Espina 1994; Jensen 1995; Neumann *et al.* 2001). The rate of chloride active uptake is hindered by high external nitrite concentrations, because NO_2^- exhibits high affinity for the Cl^- uptake mechanism (Jensen 1995, 2003). Once inside the animal, nitrite can cause dysfunctions of the oxygen-carrying pigments, leading to hypoxia and finally death (Alcaraz and Espina 1994; Jensen 1995; Philips *et al.* 2002; Camargo and Alonso 2006). The oxidation of respiratory pigments seems to be especially active in hemoglobin (fish pigment), being less important in hemocyanin (crustacean pigment) (Jensen 1995, 2003). Other physiological alterations, such as modifications in cardiac activity, reductions in extracellular chloride concentration and muscle K^+ content, hyperventilation, extracellular alkalosis, and enzymatic alterations, have also been observed in aquatic animals exposed to nitrite ions (Jensen 1995, 2003; Das *et al.* 2004; Camargo and Alonso 2006).

Because both nitrite ions and chloride ions compete for the same site of active transport, nitrite toxicity to freshwater animals may be mitigated by elevated chloride concentrations in the aquatic environment (Alcaraz and Espina 1994; Cheng and Chen 1998; Camargo and Alonso 2006). This ameliorating effect of chloride is well documented in some freshwater species, especially in fish (Lewis and Morris 1986; Tomassò 1986; Alcaraz and Espina 1994; Bartlett and Neumann 1998; Tomasso *et al.* 2003; Camargo and Alonso 2006). Similarly, it has been found that sodium and potassium can reduce ammonia toxicity to some aquatic invertebrates (Borgmann and Borgmann 1997; Beketov 2002). However, there is scarce knowledge on the toxic effects of nitrite to wild freshwater invertebrates and their interaction with chloride ions (Glass 1996; Kelso *et al.* 1999; Neumann *et al.* 2001; Alonso and Camargo 2006a). Furthermore, the ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates, lacking specific respiratory structures and pigments (*e.g.*, planarians), has been amply ignored.

The aim of this study was to compare the ameliorating effect of chloride on nitrite toxicity to two species of freshwater invertebrates differing in physiology: the amphipod *Eulimnogammarus toletanus* (Pinkster & Stock) (Gammaridae, Crustacea), and the planarian *Polycelis felina* (Dalyell) (Planariidae, Turbellaria). Amphipods bear gills and hemocyanin for oxygen transport, whereas planarians neither have specific respiratory structures nor pigments (Thorp and Covich 2001). A higher protective effect of chloride must hence be expected in amphipods than in planarians. Additionally, the Iberian amphipod *E. toletanus* has been found to be very sensitive to nitrite toxicity, whereas *P. felina* has shown a high tolerance to this inorganic nitrogen compound at relatively low chloride levels (56 ppm Cl^-; Alonso and Camargo 2006a). Nevertheless, as far as we know, the protective effect of chloride has not yet been examined in these species.

Materials and Methods

Test Organisms and Acclimatization

Invertebrates were collected from an unpolluted upper reach of the Henares River (Guadalajara province, Central Spain). Planarians were collected from the underside of stones using a soft paintbrush, and amphipods were collected with a hand-net (0.250 mm). Animals were transported to the laboratory in plastic containers filled with water from the sampling reach. Once in the laboratory, individuals of each test species were introduced into glass aquaria (1.0 L) and progressively acclimatized to test water (bottle drinking water without chlorine). In general, the Henares River water and test water showed similar physicochemical characteristics (Table 1). Additionally, four different concentrations of chloride were established in test water: the first one was a baseline mean chloride concentration of 27.8 ppm Cl^- (Table 1), and the rest were 58.3, 85.3, and 108.0 ppm Cl^- (see Experimental Design below). The acclimatization period lasted a week prior to the start of bioassays. Precopulatory pairs and gravid amphipods were rejected for the experiment. During acclimatization, amphipods were fed with stream-conditioned poplar leaves (*Populus* sp.), and planarians with gravid amphipods. No food was supplied during bioassays.

Table 1 Mean values (± SD) of physicochemical characteristics of both Henares River water in the sampling site and test water[a]

	Test water	Henares water
Conductivity (μS)	429.7 ± 17.8	536.4 ± 13.6
pH	8.0 ± 0.3	7.7 ± 0.09
Water temperature (°C)	14.3 ± 0.9	11.3 ± 1.8
Dissolved oxygen (ppm)	7.4 ± 0.2	8.7 ± 0.7
Chloride (ppm)	27.8 ± 1.2	6.2 ± 0.6
Calcium (ppm)	39.1 ± 1.6	84.4 ± 17.5
NH_3-N (ppm)	<0.002	<0.002
NO_2-N (ppm)	<0.005	<0.005
NO_3-N (ppm)	2.3 ± 0.8	1.5 ± 1.4

[a] Water analyses were performed following the standard methods of American Public Health Association (1995)

Experimental Design

An independent bioassay (168 h) was conducted in triplicate for each species using small glass vessels (capacity of 0.1 L). Test solutions and controls were renewed daily. All vessels were covered with a perforated plastic foil in order to reduce water evaporation. Eight individuals were randomly assigned to each vessel. In both bioassays, animals were exposed to one lethal concentration of nitrite at four mean concentrations of chloride (27.8, 58.3, 85.3, and 108.0 ppm Cl^-) and to two controls, with 27.8 ppm Cl^- and 108.0 ppm Cl^-. These ranges of chloride concentrations have been found in relatively unpolluted reaches of the Henares River (Alonso and Camargo 2006a). Nominal nitrite concentrations were 5.0 and 100.0 ppm NO_2-N for the amphipod and planarian bioassays, respectively, being around twice of the 96-h LC_{50} values obtained at 56 ppm Cl^- for both species (Alonso and Camargo 2006a). These test nitrite concentrations were chosen to ensure lethal effects on both species, and to test the possible ameliorating effect of different chloride concentrations on a short-term exposure to a lethal nitrite concentration in each species. Nitrite solutions for each bioassay were prepared daily from nitrite stock solutions of 100 ppm NO_2-N plus 27.8, 58.3, 85.3, or 108.0 ppm Cl^-, by dissolving the required amount of sodium nitrite (NO_2Na, Sigma, Steinheim, Germany, lot no. 97H1563, reported purity of 99.5%) in 1000 mL of chloride test water. Chloride solutions were prepared at the beginning of the bioassays by dissolving the required amount of sodium chloride (NaCl, Panreac, Spain, lot no. 142060830, reported purity of 99.0%) in 5000 mL of test water, which had a baseline concentration of 27.8 ppm Cl^- (Table 1). Both salts were weighted after drying at 60°C during 48 h. Water temperature, pH, dissolved oxygen, and chloride and nitrite concentrations were monitored daily. Actual chloride and nitrite concentrations were measured using the argentometric method and the spectrophotometric method, respectively (Spectroquant-Merk®, Germany. Detection limit = 0.005 ppm NO_2-N) (APHA 1995).

Bioassay Monitoring and Statistical Analysis

In both bioassays, two parameters were monitored every 24 h over a period of 7 days: the proportion of dead invertebrates and the proportion of affected individuals (*i.e.*, dead plus inactive invertebrates) (Newton *et al.* 2003; Alonso and Camargo 2006b). An amphipod was considered dead when neither swimming displacement nor movement of any body part were observed after touching the animal with a glass stick, and inactive when no swimming displacement was observed but some body part was active (such as pleopods, uropods, antenna, or gills). The death of a planarian was established when body tissues started to decompose. A planarian was considered inactive when body contraction and no displacement were observed after touching the animal with a soft paintbrush, but body tissues were intact. The endpoint of affected animals was chosen because it has been previously observed and considered as a useful sensitive endpoint for freshwater invertebrates (Alonso and Camargo 2006b). Dead invertebrates were removed every day.

Lethal time (LT_{50}) and effective time (ET_{50}) and their respective 95% confidence limits were calculated for each chloride concentration in each species. The multifactor probit analysis software (MPA; a software developed by U.S. Environmental Protection Agency 1991) was used to calculate LT_{50} and ET_{50} values. The time (hours) was considered as the independent variable (log transform), and the probit of the proportion of invertebrate responding to each time was the dependent variable. One of the advantages of using probit analyses is that 100% responses really are not needed to calculate LC_{50}, LT_{50}, EC_{50}, or ET_{50} values (U.S. Environmental Protection Agency 1991).

Before starting the planarian bioassay, *P. felina* body lengths were measured using a Delta-T leaf area meter (Cambridge, UK). After finishing the *E. toletanus* bioassay, the body length from antennal base to third uropod was measured for each amphipod with an ocular micrometer. Differences in body lengths between treatments were assessed through a one-way analysis of variance followed by a post-hoc Tukey test for each bioassay. LT_{50} and ET_{50} values were considered to be significantly different ($p < 0.05$) between chloride concentrations within a species when 95% confidence limits did not overlap (U.S. Environmental Protection Agency 1991; Mummert *et al.* 2003). Ratio test was conducted to assess significant differences ($p < 0.05$) between chloride concentrations within each

Table 2 LT_{50} and ET_{50} values (expressed in hours) for *Eulimnogammarus toletanus* and *Polycelis felina* after exposures to a mean actual concentration of 5.1 and 101.3 ppm NO_2-N, respectively, at four different mean actual concentrations of chloride*

	Eulimnogammarus toletanus		*Polycelis felina*	
Chloride (ppm)	LT_{50} (h)	ET_{50} (h)	LT_{50} (h)	ET_{50} (h)
27.8	45.1[a] (36.8–53.0)	<24¶	36.5[a] (29.7–44.4)	<24¶
58.3	88.1[b] (73.4–104.2)	51.1[a] (42.1–43.2)	47.9[b] (37.3–57.8)	<24¶
85.3	135.1[c] (114.0–179.6)	97.9[b] (83.0–115.6)	71.1[c] (61.9–79.6)	<24¶
108.0	148.0[c] (129.0–187.4)	127.1[c] (113.6–144.9)	72.1[c] (60.9–83.4)	<24¶

¶ ET values could not be calculated

* LT_{50} and ET_{50} values were calculated using the multifactor probit analysis software (MPA) software (US Environmental Protection Agency 1991). The time (hours) was considered as the independent variable (log transform), and the probit of the proportion of individuals responding to each time was the dependent variable. Letters (a, b, c) indicate significant differences between chloride concentrations for each LT_{50} or ET_{50} value. 95% confidence limits are presented in parentheses

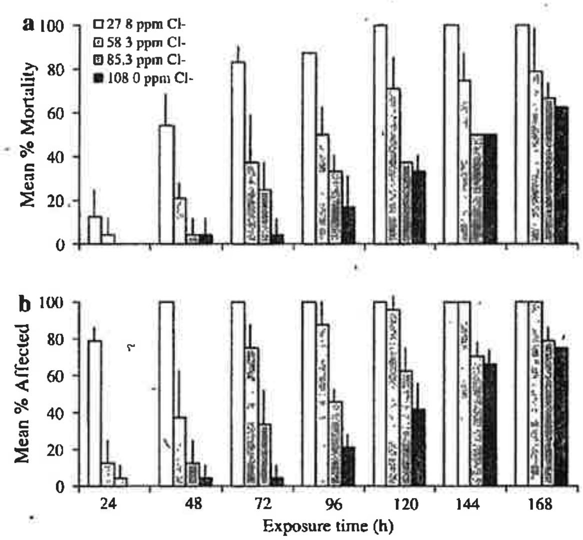

Fig. 1 Mean percentages (+SD) of mortality (**a**) and affected individuals (**b**) for *Eulimnogammarus toletanus* exposed to 5.1 ppm NO_2-N through seven different exposure times (hours) and at four different chloride concentrations (ppm Cl^-)

species when LT_{50} and ET_{50} confidence limits overlapped (Wheeler *et al.* 2006). A regression analysis between chloride concentrations (independent variable) and LT_{50} values (dependent variable) was conducted for each species to elucidate the possible ameliorating effect of chloride.

Results and Discussion

All animals in control vessels (27.8 and 108.0 ppm Cl^-, and <0.005 ppm NO_2-N) survived after 7 days. Inactive animals in these controls were lower than 10% for amphipods and 0% for planarians. Mean body lengths were 6.4 ± 1.0 mm for planarians and 5.6 ± 0.8 mm for amphipods, with no significant difference being found among treatments (including controls) ($p > 0.05$; Tukey test). Mean actual concentrations of nitrite (ppm NO_2-N) in amphipod and planarian bioassays were 5.1 ± 0.1 (n = 14) and 101.3 ± 1.9 (n = 14), respectively. The mean mortality and the proportion of affected animals for *E. toletanus*, at each environmental chloride concentration and exposure time, are shown in Figure 1, and the mean mortality for *P. felina* is shown in Figure 2. LT_{50} and ET_{50} values, and their respective 95% confidence limits, are presented in Table 2 for each test species and each chloride concentration. In both test species, LT_{50} values increased in parallel to the environmental chloride concentration (Figure 3), being significantly different between chloride concentrations ($p < 0.05$; 95% confidence limits did not overlap), except for the two highest concentrations in which they did not significantly differ ($p > 0.05$; Ratio test). ET_{50} values for *E. toletanus* at 27.8 ppm Cl^- could not be calculated, because more than 50% of individuals were affected after 24 h of exposure to 5.1 ppm NO_2-N (Figure 1). The rest of ET_{50} values paralleled the trend of LT_{50} values (Table 2), except for the two highest concentrations in which they differed significantly ($p < 0.05$; Ratio test). ET_{50} values for *P. felina* could not be calculated, because all individuals were inactive after 24 h of exposure to 101.3 ppm NO_2-N at all chloride concentrations (data not shown). The regression analysis between the environmental chloride concentrations and LT_{50} values was positive and significant for both test species, with R^2 values being higher than 0.90 (Figure 3).

The present investigation has clearly shown that chloride ions may protect freshwater invertebrates against nitrite toxicity. This ameliorating effect of chloride has been previously found in the amphipod *Gammarus* sp., in which an artificial supplement of chloride (70 ppm Cl^-) reduced its mortality, and in the dipteran *Chironomus* sp., in which chloride reduced the adverse effects of nitrite on

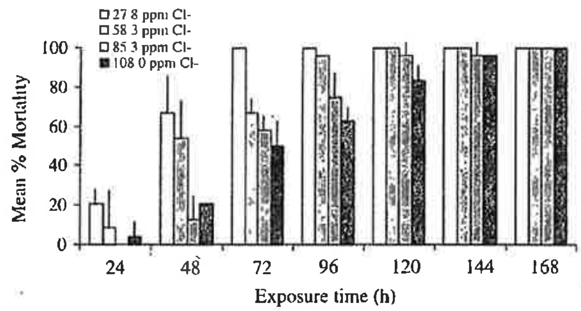

Fig. 2 Mean percentages (+SD) of mortality for *Polycelis felina* exposed to 101.3 ppm NO_2-N through seven different exposure times (hours) and at four different chloride concentrations (ppm Cl^-)

the development of its anal papillae (Glass 1996; Kelso *et al.* 1999; Neumann *et al.* 2001). The Cl^-/NO_2^- competition system is believed to be operational in *Gammarus* sp., and in the case of *Chironomus* sp. it seems to be associated with the anal papillae. Furthermore, Camargo (2004) found that chloride ions increased the tolerance of aquatic larvae of the net-spinning caddisfly *Hydropsyche tibialis* to fluoride toxicity, suggesting that this ameliorating effect was the result of competition between F^- and Cl^- ions for the same active transport site. In this connection, our regression analysis (Figure 3) shows that chloride ions reduced significantly the short-term toxicity of nitrite ions in two contrasting species of freshwater

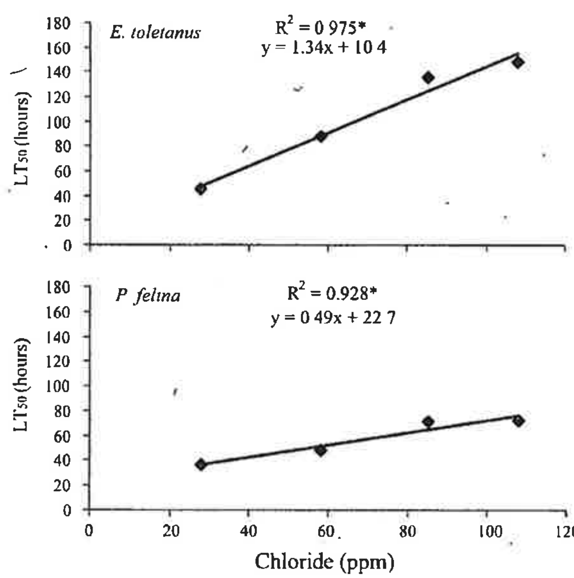

Fig. 3 Regression analysis between LT_{50} values (hours) and chloride concentrations (ppm Cl^-) for *Eulimnogammarus toletanus* (exposed to 5.1 ppm NO_2-N) and *Polycelis felina* (exposed to 101.3 ppm NO_2-N) Asterisk indicates a significant linear relationship between both variables ($p < 0.05$)

invertebrates, and consequently the results might be used to extrapolate chloride effects to other related freshwater invertebrates.

There is strong evidence that the gills are the organs of active ion transport in several freshwater groups, including crayfish and amphipods (Barnes *et al.* 1993; Thorp and Covich 2001). In addition, the respiratory singular pigment in these animal groups is hemocyanin (Ruppert and Barnes 1994). By contrast, planarians have no specific respiratory structures and pigments, the gas exchange being performed by simple diffusion through their body walls (Barnes *et al.* 1993; Ruppert and Barnes 1994; Kolasa 2001). In the absence of specific respiratory structures and pigments, the uptake of Cl^- and NO_2^- across the body walls appears as a likely possibility in planarians (Kolasa 2001). The uptake of NO_2^- might be both via active and passive transport. Indeed, with the highest environmental nitrite concentration used in the present study, passive transport could be an important way for uptake, even if its permeability is low. The latter issue could also explain the high tolerance of planarians to the nitrite toxicity.

Our results highlight that nitrite safe levels in the aquatic environment may be higher the higher the chloride concentrations. This fact seems to be especially relevant for assessing the environmental impact of industrial and sewage effluents, because they often introduce high amounts of both chloride ions and inorganic nitrogen compounds into the recipient freshwater ecosystems (Meybeck *et al.* 1989; Gleick 1993; Smil 2001; Orozco *et al.* 2003). Overall, we conclude that the presence of high chloride concentrations in the aquatic environment may cause an ameliorating effect on nitrite toxicity to the two test freshwater invertebrate species, particularly regarding the sensitive amphipod *E. toletanus*. Therefore, the influence of chloride ions on the toxicity of nitrite ions must be taken into account when the ecotoxicological risk assessment of nitrite is performed in freshwater ecosystems. A similar recommendation was pointed out by Camargo (2004) with regard to the real risk of fluoride ions for freshwater invertebrates. However, it would be necessary to get further information on the ameliorating effects of chloride ions on long-term nitrite toxicity to freshwater invertebrates and other animals in order to determine more realistic nitrite safe levels with different environmental chloride concentrations.

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